WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 5, Issue 2, 1153-1161. Research Article ISSN 2277– 7105

EFFICIENT ULTRASOUND SYNTHESIS AND BIOLOGICAL SCREENING OF METAL COMPLEXES OF N-(4-METHOXYBENZYLIDENE) ISONICOTINOHYDRAZONE

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Article Received on
06 Dec 2015,

Revised on 27 Dec 2015,
Accepted on 16 Jan 2016

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ABSTRACT

Here we report the synthesis of three new complexes of Ni(II), Zn(II) and Co(II) with the ligand N-(4-methoxybenzylidene) isonicotinohydrazide, having the formula $[M((L_A))(ac)_2]xH_2O$ (M = Ni(II), Zn(II) and Co(II)) with their characterization. The ligands were synthesized by the condensation of isonicotinic acid hydrazide with 4-methoxybenzaldehyde. The complexes were synthesized by using ultrasound irradiation method and were characterized by elemental analysis, FTIR, UV-Vis spectroscopy, molar conductivity and evaluated for their in vitro antibacterial activity against human pathogens like gram negative Escherichia coli (E.coli), Salmonella Typhi (S. typhi) and gram positive Bacillus Subtilis (B. subtilis), Staphylococcus aureus strains. The result indicates that in all complexes, ligand was coordinated to metal via N and O donor and having moderate antimicrobial activity.

KEYWORDS: 4-methoxy benzaldehyde, isoniazid, ultrasound irradiation method, transition metal complexes, human pathogens, antimicrobial activity.

INTRODUCTION

Developing metal-based drugs is emerging as an active area of research. Schiff base metal complexes have been amongst the most widely studied coordination compounds, this is due to the fact that Schiff bases offers opportunities for alteration the metal centered electronic factor, enhancing the stability and solubility[1-4] and are active against a wide range of organisms such as Staphylococcus aureus, Escherichia coli, Bacillus polymxa, Mycobacteria, Plasmopora viticola and Trychophyton gypseum.[5]

There has been growing interest in the study of hydrazones because of their physiological activity, coordinative capability and applications in analytical chemistry.[6-8] Compared to the simple hydrazone Schiff bases, aroyl or heteroaroyl Schiff bases have additional donor sites. This introduces a wider range of properties like antibacterial, antimycobacterial, anticonvulsant, analgesic, anti-inflammatory and antitumor activities.

The transition metal complexes are far more biologically active than uncoordinated hydrazones and their enhanced biological activity has been an active area of investigation among medicinal researchers,[9-11] these coordination compounds of arylhydrazones have been reported to acts as enzyme inhibitor.[12] In general, arylhydrazones R-CO-NH-N=CHR as chelating ligands with transition metal ions by binding through the N atom of azomethine group and O atom of carbonyl group and therefore this type of compounds can coordinate *in vivo* to metal ions.

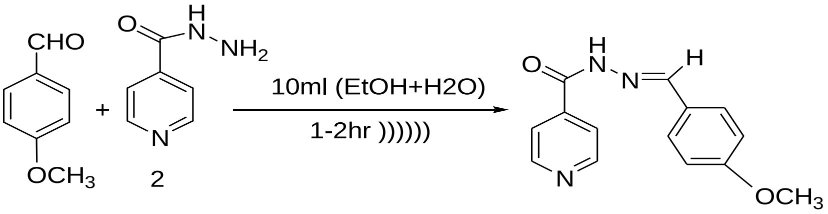
The use of ultrasound in organic transformation is now well known to enhance reaction rates and yields/selectivity of reactions and in several cases facilitates organic transformation at ambient conditions.[13] Many homogeneous and heterogeneous reactions can be conducted smoothly by sonication. Therefore organic reaction carried out by using ultrasound irradiation is an efficient and eco-friendly synthetic strategy.

Taking into account these antecedents and as part of our work in the research of new antimicrobial molecules,[14] here in this paper we report the synthesis of three new complexes of Ni(II), Zn(II) and Co(II) with the ligand N-(4-methoxybenzylidene)isonicotinohydrazone by using ultrasound irradiation along with their characterization and biological activity study.

EXPERIMENTAL

All used chemicals were purchased from Merck or S.D. Fine chemicals. Melting points were determined on an electrochemical digital melting point apparatus. The formation of the product was determined by thin layer chromatography. Products were characterized by comparison of spectroscopic data (UV-Visible, FT-IR, ^{1}HNMR spectra) and melting points with authentic samples. The wavelength of absorbance was determined by UV-Visible spectrophotometer [JASCO 503] using a quartz quvette and ethanol as reference. The IR spectra were recorded on FT-IR spectrophotometer [JASCO, FT-IR/4100] Japan using dry KBr as standard reference. ^{1}H NMR and ^{13}C NMR spectra were recorded on an Broker Avance II 400 MHz FT-NMR spectrometer in DMSO as a solvent and chemical shift values are recorded in units δ (ppm) relative to tetramethylsilane (Me_4Si) as an internal standard. Elemental analyses were done using Perkin Elmer 2400CHN analyzer. The magnetic susceptibility of the complexes was measured at room temperature using a Gouy balance.

General procedure for the synthesis of N-(4-methoxybenzylidene) isonicotinohydrazone: For the synthesis of ligand (L_A) INH (10.0mmol) was dissolved in 10 ml of ethanol+H_2O (6:4) stir for some time to give a clear solution to this 4-methoxy benzaldehyde (10.0mmol) was added drop wise over 5 min. The resulting reaction mixture was irradiated for an appropriate time under ultrasound. After completion of the reaction (monitored by TLC) it was allowed to cool slowly and stand overnight. Obtained crystalline solid was filtered off, washed with hot water and dried. It was recrystallized from ethanol. Yield: 92%; M. P.: 138-140°C.



Scheme1: Synthesis of Schiff base ligand L_a using ultra sound irradiation

FT-IR (KBr disk, ν_{max}): 3446.7 (CO-NH-), 3207.76 (-NH), 3039.8 & 2993.5 (C-H, aromatic), 1689.5 (C=O), 1649.4 (C=N), 1599.2 (C=C), 1227.2 (C-O)cm^{-1}; ^{1}H-NMR(DMSO-400MHz) δ=12.321 (s,2H,CO-NH-), 8.813 (s,2H,CH=N), 7.165-8.043 (m,8H,Ar); ^{13}C NMR (DMSO-400MHz) δ= 163.23, 160.32, 149.72, 149.84, 143.51, 141.11, 132.23, 130.26, 122.89, 122.87, 121.72, 116.13, 114.58, 55.92; UV/Vis(DMSO)nm: 286nm and 368nm, Anal Calcd for $C_{14}H_{13}N_3O_2$: C, 65.88. H, 5.09. N, 16.47%. Found: C, 65.83. H, 5.12. N, 16.44%.

Experimental procedure for synthesis of complexes

To the warm methanolic solution (10 mL) of ligand L_A (2mmol), 10ml warm methanolic solutions (1mmol) of acetate salts of metal (Zn(II), Co(II) and Ni(II)) was added and resulting mixture was irradiated for about 1-3 hrs. The obtained precipitates were filtered, washed with methanol and dried under vacuum on anhydrous $CaCl_2$.

Antimicrobial activity

The ligand (L_A) and its metal complexes were screened for in vitro antimicrobial activity in DMSO against gram negative *Escherichia coli (E.coli) Salmonella Typhi (S.typhi)* and gram positive *Bacillus Subtilis (B. subtilis), Staphylococcus aureus* strains by Kirby Baur's disc diffusion technique. A uniform suspension of test organism of 24 hours old culture was prepared in test tube containing sterile saline solution. A sterile nutrient agar was then added in each of the Petri dishes. The dishes were related to ensure the uniform mixing of the micro organism in the agar medium which was then allowed to solidify. Sterile Whatmann filter paper discs were dipped in the solution of each compound and placed on the labeled plates. The DMSO was used as control of the solvent. Gentamicine was used as a standard compound for comparison. Plates were kept in refrigerator for half an hour for diffusion and incubated at 37°C for 24 hours. The diameter of zone of inhibition around each disc was measured by scale and results were recorded in terms of mm. The observed data of antimicrobial activity of compounds and the standard drugs are given in Table 3.

RESULT AND DISCUSSION

In the present study it was observed that the reaction under ultrasound irradiation had significantly improved the yield of product. By the reaction of Zn(II), Co(II) and Ni(II) acetate with ligand L_A, complexes of type $[M(INOMeH)(ac)_2]xH_2O$ was obtained. All the complexes have different color, stable at room temperature, insoluble in common polar solvent but soluble in DMSO and DMF, do not have sharp melting point but decompose above 290°C.

The elemental analysis is in good agreement with the chemical formula proposed for complexes. The measurement of molar conductivities at 10^{-3}M concentration carried out in DMSO at room temperature. The lower conductance value supports the non-electrolytic nature of the complexes.[15] The analytical and physical data (color, melting point and molar conductivity) of the complexes are given in Table 1. Magnetic moments of the complexes were measured at room temperature and the values are given in Table 2. For the Co (II) complexes the magnetic moments were 5.12BM, indicating a high-spin character. This value corresponds to an octahedral geometry.[16, 17] For the Ni(II) complex the value for the magnetic moments is 2.80BM indicates the paramagnetic complex of Ni(II) with octahedral geometry. The Zn(II) complex is diamagnetic, as expected for complexes of metal ions with a d^{10} configuration.

Table 1: Analytical and physical data of the complexes

Compound	Color	Yield (%)	M. P. (°C)	$\Lambda^a/\Omega^{-1}cm^2 mol^{-1}$	Elemental analysis Found (Calcd.)			
					%C	%H	%N	%M
$C_{14}H_{13}N_3O_2(L_A)$	Light Yellow	92%	138-140	---	65.83 65.88	5.12 5.09	16.44 16.47	---
$[Co(L_A)(ac)_2]\cdot 4H_2O$	Brown	81%	290	6.23	42.33 42.39	4.09 4.13	9.16 9.27	12.69 13.01
$[Ni(L_A)(ac)_2]\cdot 2H_2O$	Faint orange	85%	>300	8.12	47.18 47.21	4.46 4.43	10.39 10.33	14.45 14.43
$[Zn(L_A)(ac)_2]\cdot 2H_2O$	Yellow	88%	269	7.79	46.49 46.45	4.31 4.35	10.12 10.16	15.83 15.82

$^a10^{-3}$M solution in DMSO

IR Spectral Studies

The IR spectra of complexes were compared with those of free ligand and previously reported complexes[18-20] and selected IR spectral band for the ligand and its metal complexes are given in Table 2. In the IR spectrum of ligand the ν C=O band appeared at 1689.5cm^{-1} and ν C=N at 1649.4 cm^{-1}.The band due to free –NH group appeared at 3446.7 and 3207.76cm^{-1} which remain unaffected after complexation.[21] In complexes a shift to words lower values by 31-40 cm^{-1} for the band characteristic to azomethine group indicating the involvement of the azomethine nitrogen in the coordination with the metal ions. The band corresponding to amide I was shifted by 21-53 cm^{-1} to words lower frequency, which indicates the involvement of carbonyl group in the coordination. The overall IR spectra suggest that ligand acts as bidentate ligand. IR spectra of complexes shows two absorption bands at 1492 cm^{-1} and 1349 cm^{-1}, assigned to antisymmetric and symmetric acetate group frequencies. The difference $\Delta\nu$ (ν_{as}-ν_{sym}) suggest that the acetate group in these complexes was in a chelating binding mode.[22] Along with these IR spectra of the complexes exhibited new non-ligand bands in the range 496-589 cm^{-1} assigned as M-O and M-N stretching vibrations,[23,24] respectively. Therefore, it can be concluded that (L_A) ligand binds to the metal ions through azomethine N and the carbonyl O atom.

Table 2: Key infrared bands (cm^{-1}) of complexes and ligand L_a

Compound	ν(-OH)	ν(-NH)	ν(C=O)	ν(C=N)	ν(M–N)	ν(M–O)	μ_{eff}(BM)
$C_{14}H_{13}N_3O_2(L_A)$	-	3446.7 3207.76	1689.5	1649.4	-	-	
$[Co(L_A)(ac)_2]\cdot 4H_2O$	3415.1	3442.2 3198.5	1661.4	1598.2	492	410	5.06
$[Ni(L_A)(ac)_2]\cdot 2H_2O$	3451.1	3439.2 3189.2	1649.3	1584.5	520	462	2.80
$[Zn(L_A)(ac)_2]\cdot 2H_2O$	3412.3	3444.4 3180.5	1656.2	1590.2	514	425	Diamagnetic

Electronic Absorption Spectra and Magnetic Moments of the Metal Complexes

The UV-Vis spectra of the ligand shows two bands at 286 and 368nm which are assigned to π-π* and n-π* transition respectively. The UV-Vis spectra of 10^{-4}M of the metal complexes showed similar absorption spectra of the ligand which are shifted to lower and higher wavelengths beside a decrease or disappearance of the peak dye to n-π* transition which confirm the coordination through azomethine nitrogen. Also the d-d transition in this type of complexes may appear above 500 nm but does not appear due to the low intensity of the d-d transition.

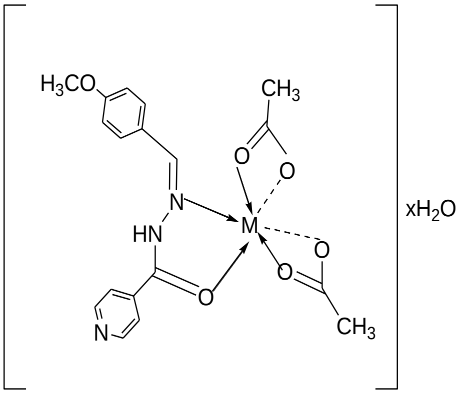


Figure 1: The suggested geometry of Co(II), Ni(II) and Zn(II) Chelate complexes for La

Antimicrobial screening Result

The Schiff-base ligand and its metal complexes reported here were evaluated for antibacterial activity against *Escherichia coli*, *Salmonella Typhi*, *Bacillus subtilis* and *Staphylococcus aureus*. The values of zone inhibition were measured in millimeter. The data of antibacterial activities of ligand and complexes are in give in (Table 3, Figure 2). The inhibitory zone data reveals that all ligands as well as their metal complexes show good to moderate antibacterial activity. The biologically activity of Schiff base ligand arise from the presence of imine group which imports in elucidating the mechanism of transformation reaction in biological systems. However its metal complexes showed remarkable antibacterial activity as a result of chelation of metal with organic ligand synergistically increasing its effect.[25] The DMSO control did not show any antimicrobial activity against the tested bacterial strains while considering with respect to standard, all the tested compounds were found to be moderately active.

Such increased activity of the metal chelates can be explained on the basis of Overtone's concept and Tweedy's chelation theory.[26] According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials due to which liposolubility is considered to be an important factor that controls the antibacterial activity. On chelation, there is reduction in the polarity of the metal atom by the partial sharing of its positive charge with donor groups and possible π–electron delocalization over the whole ring. This in turn, increases the lipophilic character of the metal chelate and favours its permeation through the lipid layers of the membranes of the micro-organism and thus blocks the metal binding sites on enzymes of microorganisms.[27-29] Apart from this, other factors such as solubility, conductivity and dipole moment influenced by the presence of the metal ions may also be reasons for the increased activity.

Table 3: Antibacterial screening results of ligand L_A and its complexes

Antibacterial zone of inhibition (in mm)				
	Gram positive		**Gram negative**	
Compound	***B. subtilis***	***S. aureus***	***S. typhi***	***E.coli***
L_A	6	7	8	8
Co- L_A	18	17	12	16
Ni- L_A	11	10	12	11
Zn- L_A	13	13	12	11
Gentamicine	10	8	9	8
DMSO	0	0	0	0

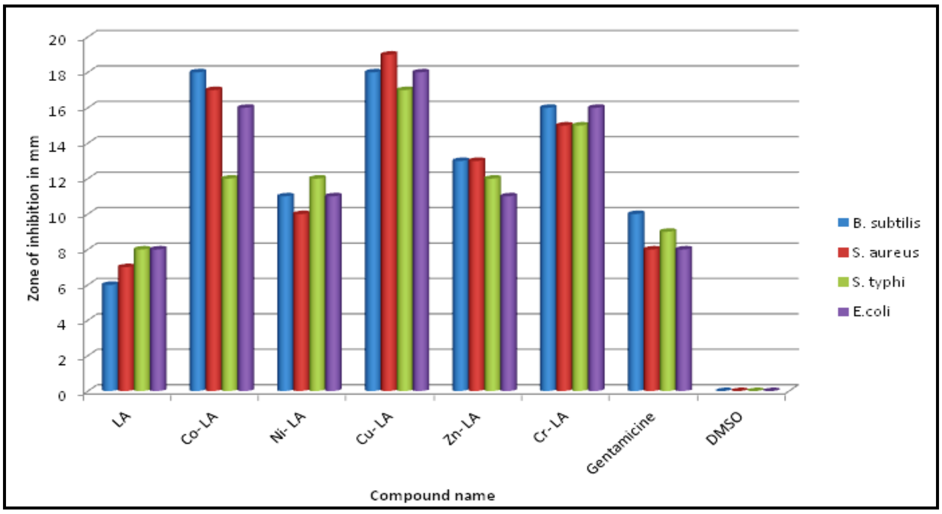


Figure 2: Antibacterial screening result of ligand L_A and its complexes

CONCLUSION

The synthesized compounds are active against all bacteria. It has been observed that all metal complexes show more antibacterial activity than ligand. Although with respect to standard, all the tested compounds were found to be moderately active. In summary, this work demonstrated a rapid, efficient and environmentally friendly method for the synthesis of INH hydrazide-hydrazones metal complexes under ultrasound irradiation and results obtained confirmed the superiority of ultrasound irradiation method over the conventional method.

ACKNOWLEDGMENTS

The authors are grateful to the Head, Department of Chemistry, J.E.S. College Jalna and Dept. of Chemistry, Govt. College of Arts & Science, Aurangabad for providing the laboratory facility.

REFERENCES

1. Unaleroglu C, Temelli B, Hokelek T, J. Mol. Struct., 2001; 570: 91.
2. Golcu A, Tumer M, Demirelli H, Wheatley RA, J Inorganica Chimica Acta, 2005; 358: 1785-1797.
3. Opstal T, Verpoort F, Angew. Chem. Int Ed. 2003; 42: 2876.
4. Mohapatra RK, Ghosh S, Naik P, Mishra SK, Mahapatra A and Dash DC, J. Korean Chem., Soc, 2012, 56(1): 62-67.
5. T¨umer M, K¨oksal H, S¸ ener MK, Serin S, Trans. Met. Chem., 1999; 24(4): 414–420.
6. Rastogi DK, Sahni SK, Rana VB, J. Coord. Chem., 1978; 8: 97.
7. El-Bahnasawy RM, El-Meleigy SE, El-Tawansi A, Trans. Met. Chem., 1994; 19: 270.
8. Naran KK, Singh VP, Synth. React. Inorg. Met.-Org. Chem., 1997; 27: 721.
9. Maurya RC, Verma R, Singh T, Synth. React. Inorg. Met.-Org. Chem, 2003; 33: 309.
10. Singh NK, Singh N, Prasad GC, Sodhi A, Shrivastava A, Bioorg. Med. Chem., 1997; 5: 245.
11. Prasad S and Agarwal RK**,** J Korean Chem. Soc., 2009; 53(1): 17-26.
12. Kakimoto S, Yamamoto K, Pharm. Bulletin, 1956; 4(1): 4–6.
13. (a) Rajagopal R., Jarikote DV, Srinivasan KV, Chem. Commun., 2002; 616. (b) Song BA, Zhang GP, Yang S, Hu DY, Jin LH, Ultra. Chem., 2001; 13: 1544.
14. Kulkarni GS, Anandgaonker PL, Janrao SD, Gaikwad DD, Janrao DM, Int. J. Chem. Pharm, Sci., 2015; 3(7): 1832-1835.
15. Geary WJ, Coord. Chem. Rev., 1997; 7: 81.
16. Agarwal RK, Sharma D, Shing L, Agarwal H, Bioinorg. Chem. Appl., 2006; (2006): 29238.
17. Nakamoto K, Infrared spectra of inorganic and coordination compounds, 2nd ed., Wiley--Interscience, New York, USA, 1970.
18. Deacon GB, Philips RJ, Coord. Chem. Rev, 1980; 23: 227.

19. Agarwal RK, Sarin, RK, Polyhedron, 1993; 12: 2411.
20. Agarwal RK, Sarin RK, Agarwal H, Bull. Chem. Soc. Ethio., 1995; 9: 23.
21. Curti NF, J. Chem. Soc.**,** 1968, A: 1579.
22. Burns GR, Inorg. Chem., 1968; 7: 272.
23. Mostafa MH Khalil, Ismail EH, Mohamed GG, Zayed EM, Badr A, Open Journal of Inorg. Chem, 2012; 2: 13-21.
24. Chohan ZH and Shad HA, J. Enz. Inhib. Med. Chem., 2008; 23: 369.
25. Vogt RL, Dippoid, Public Health Res., 2005; 120: 174.
26. Anjanayalu Y, Rao PP, Synth. React. Inorg Met-Org Chem., 1986; 16: 257.
27. Chandra S, Jain D, Sharma AK; Sharma P, J. Molecules, 2009; 14: 174-190.
28. C¸ olak AT, C¸ olak F, Yesilel OZ, Akduman D, Yilmaz F, T¨umer M , Inorg.Chimica Acta, 2010; 363(10): 2149–2162.